1828-Pos Board B598
Cardiomyopathy - Associated W4R Variant of Muscle LIM Protein affects Skeletal Muscle Passive Mechanics
Ina Stehle[1], Gudrun Brandes[2], Cornelia Geers-Knörr[1], Ralph Knöll[3], Bernhard Brenner[1], Theresia Kraft[1].
[1]Molecular and Cell Physiology, Medical School, Hannover, Germany, [2]Cell Biology, Medical School, Hannover, Germany, [3]Myocardial Genetics, National Heart & Lung Institute, Imperial College, South Kensington Campus, London, United Kingdom.
Muscle LIM protein (MLP) is located at the Z-disc and the M-line of the sarcomere of striated muscle. It has multiple protein interaction partners. The MLP-W4R variant was shown to be associated with cardiomyopathy in humans. MLP-W4R knock-in mice showed myopathic changes in skeletal muscle and other mutations of MLP were associated with a skeletal myopathy in affected human individuals. Therefore, we investigated the influence of MLP-W4R on skeletal muscle function and ultrastructure.
Skinned single muscle fibers of M. vastus lat. of wildtype and MLP-W4R knock-in mice were used to characterize passive and active parameters like relaxed fiber stiffness, passive force-sarcomere length curve, isometric force generation, rate constant of force redevelopment and maximal shortening velocity. Thin sections of the muscle fibers were analyzed by transmission electron microscopy.
In comparison to wildtype, MLP-W4R muscle fibers showed a significant decrease in relaxed fiber stiffness and passive force-sarcomere length curve, whereas no significant changes occurred in isometric force generation, force redevelopment, and shortening velocity. Ultrastructurally, the alignment of myofilaments within the sarcomere was normal with MLP-W4R, however, Z-discs and M-lines were broadened.
MLP-W4R knock-in mice exhibit discrete morphological changes of skeletal myofibers consistent with a skeletal myopathy. The disturbed passive properties of the muscle fibers support the hypothesis that the MLP-W4R variant contributes to the disorder.

1829-Pos Board B599
Elastic Proteins in the Flight Muscle of Manduca Sexta
Chen-Ching Yuan, Jiangmin Liu, Thomas C. Irving.
Illinois Institute of Technology, Chicago, IL, USA.
Unlike the asynchronous flight muscles of *Lethocerus* or *Drosophila*, the flight muscles of the Hawkmoth *Manduca* sexta are synchronous, requiring a neural spike for each contraction. While the asynchronous muscles can only extend a few percent, *Manduca* flight muscle can reversibly extend 50% or more. Together with the observation that length-tension curves of *Manduca* flight muscles resemble mammalian cardiac muscle, these observations suggest that Manduca muscle might be a useful model system to study some aspects of cardiac muscle contractility. The detailed protein composition of *Manduca* flight muscle is not known. Here we aimed to identify proteins which might be responsible for the unique properties of *Manduca* muscle. We used 1% vertical SDS-agarose gel electrophoresis (VAGE) to separate the high molecular weight proteins in Maduca flight muscle, Lethocerus flight muscle and bovine ventricular myocardium. The *Manduca* sample showed two bands around 2MDa and 1.6MDa, smaller than the two titin isoforms in bovine cardiac muscle, but larger than the largest *Lethocerus* proteins. Projectin and Kettin are elastic proteins found in *Lethocerus* and *Drosophila* with sequence homologies to vertebrate titin. Using western blots, the Manduca sample showed two bands cross-reacting with projectin antibodies at ~800 kDa and ~1030 kDa. Kettin antibodies also cross-reacted to bands at the same position in both *Lethocerus* and *Manduca*. We also used western blots from 10% PAGE gels to detect a flightin -crossreacting band at around 23kDa in *Lethocerus* and 30 kDa in *Manduca*. Flightin is a thick filament associated protein that presumably helps filament assembly and stability. Thus, *Manduca* flight muscle has not only proteinshomologous to *Lethocerus* projectin, kettin, flightin, but also several unknown high molecular weight proteins which might play a role in stabilizing sarcomere structure. Supported by NSF IOS 1022058 and NIH RR08630.

1830-Pos Board B600
Role of Electrostatics in the Interactions of Muscle Thick Filament Proteins
Larissa Tskhovrebova, Sarika Khasnis, John Trinick.
University of Leeds, Institute of Molecular and Cellular Biology, Astbury Centre for Structural Biology, Leeds, United Kingdom.
Electrostatic interactions play a primary role in the assembly and stability of muscle thick filaments. This is illustrated by the solubility properties of the filament and its periodic structure, which correlates with the periodic distribution of positively and negatively charged residues in the sequence of light meromyosin (LMM), the main component of the filament core. In this work, we analysed the electrostatic fields of proteins associated with LMM in thick filaments, titin and MyBP-C, to explore further their role in filament assembly. The thick filament bound part of titin consists mainly of immunoglobulin (Ig) and fibronectin (Fn3) domains arranged in repeated patterns, or super-repeats, which size correlates with the periodic structure of the filament. Analysis of the domain electrostatics suggests periodic oscillations of the field along the super-repeats, with a major interval of ~43 nm and a weaker (less consistent) interval of ~14 nm. These oscillations result from the mostly opposing electrostatic fields from the Ig and Fn3 domains, respectively, and correlate with the periodic structure of the filament.
Analysis of the electrostatic fields of the Ig and Fn3 domains of cardiac MyBP-C reveals three distinct regions: the N- (C0-C2) and C-terminal (C8-C10) groups of domains are positive or neutral, whereas the central domains (C3-C7) are mainly negative. Further analysis of binding partners of MyBP-C, including titin and LMM, illustrates general complementarity of the overall electrostatic fields of the interacting proteins, thus directly supporting the involvement of electrostatics in the interactions between all the major thick filament proteins.

1831-Pos Board B601
Structure of Titin PEVK Explored with FRET Spectroscopy
Tamas Huber[1], Livia Fulop[2], Laszlo Grama[1], Csaba Hetenyi[3], Botond Penke[2], **Miklos S.Z. Kellermayer**[4].
[1]University of Pecs, Pecs, Hungary, [2]University of Szeged, Szeged, Hungary, [3]Eotvos Lorand University, Budapest, Hungary, [4]Semmelweis University, Budapest, Hungary.
The PEVK domain of the giant muscle protein titin is largely responsible for molecular extensibility within the physiological sarcomere-length range. Although the domain is thought to be an intrinsically unstructured random-coil structure, several observations suggest that it may not be completely devoid of internal interactions and structural features. To test the validity of random polymer models for PEVK, here we measured the contour-length scaling of the equilibrium mean end-to-end distance of synthetic PEVK peptides with FRET (Förster's Resonance Energy Transfer) spectroscopy. Mean end-to-end distances of an 11- and a 21-residue PEVK peptide were calculated from the energy transfer efficiency between an intrinsic tryptophan donor and a synthetically added IAEDANS acceptor positioned on the N- and C-termini, respectively. We find that the contour-length scaling of mean end-to-end distance deviates from the square-root law predicted for a purely statistical polymer chain. Addition of guanidine-HCl increased, whereas the addition of salt decreased the mean end-to-end distance, indicating that both H-bonding and electrostatic interactions play role in stabilizing PEVK structure. Increasing temperature between 5-50 °C resulted in monotonous increase in FRET efficiency, suggesting that PEVK may pass through multiple conformational states separated by small energy barriers. Simulations suggest that the residual structures are loose helical configurations. Varying the distribution across these states may tune the apparent local elasticity along the PEVK domain and modulate the dynamics of passive muscle force generation.
Supported by a grant from the Hungarian National Development Agency, TAMOP-4.2.1.B-09/1/KMR-2010-0001.

1832-Pos Board B602
Stretching of Twitchin Kinase
Johan A. Strumpfer[1,2], Eleonore von Castelmur[3], Barbara Franke[4], Sonia Barbieri[4], Julijus Bogomolovas[5], Hiroshi Qadota[6], Petr Konarv[7], Dmitri Svergun[7], Siegfried Labeit[8], Klaus Schulten[1,2], Guy M. Benian[6], Olga Mayans[4].
[1]University of Illinois at Urbana Champaign, Urbana, IL, USA, [2]Beckman Institute, Urbana, IL, USA, [3]Institute of Integrative Biology, University of Liverpool, Liverpool, IL, USA, [4]Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom, [5]Universitätsmedizin Mannheim, Mannheim, Germany, [6]Department of Pathology, Emory University, Atlanta, GA, USA, [7]European Molecular Biology Laboratory, Hamburg, Germany, [8]Department for Integrative Pathophysiology, Universitätsmedizin Mannheim, Mannheim, Germany.
The giant proteins from the titin family, that form cytoskeletal filaments, have emerged as key mechanotransducers in the sarcomere. These proteins contain a conserved kinase region, which is auto-inhibited by a C-terminal tail domain. The inhibitory tail domain occludes the active sites of the kinases, thus preventing ATP from binding. It was proposed that through application of a force, such as that arising during muscle contraction, the inhibitory tail becomes detached,